INVESTIGATIONS OF POLYSACCHARIDES IN THE SOLID STATE BY ^{13}C CP-MAS NMR SPECTROSCOPY

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An brief introduction into the ^{13}C CP-MAS methodology and a review of the recent application of this technique to the study of polysaccharides is presented.

1. INTRODUCTION

For many years the NMR spectroscopist has been frustrated with his inability to study the physical properties of high molecular weight polymers such as polysaccharides because of their insolubility. To circumvent these problems, often times the materials would have to be chemically modified, e.g., acetylated, hydrolized, or enzymatically cleaved to give smaller fragments. Inevitably the substances derived from these various treatments bear little or no resemblance in dynamic properties to the original intact polymers. While X-ray crystallography has been used extensively for characterizing such polymers as cellulose, the interpretation of these results has been less than unambiguous[1]. In addition, because of the time constant associated with the crystallographic methodology only marginal dynamic information can be obtained.

In contrast, high resolution ^{13}C solid state cross polarization magic angle spinning NMR spectroscopy can be used to examine the structures of bulk polysaccharide polymers and derived in their native states. Measurement of relaxation processes can give information concerning the intimacy (domain sizes) of coexisting

polymers in such structurally diverse polymers as wood cellulose. In addition this methodology can also pinpoint the orgin of crystalline and amorphous region in the matrices as well as establish the nature and asymmetry of the polymer repeat units.

1.1 Solid State Methodology

Unlike the solution state, intermolecular interactions in the solid state are very severe. In general such interactions are dipolar in nature and manifest themselves by giving rise to broad resonance lines in the ^{13}C NMR spectra of these materials. Without the removal of these strong forces (primarily with protons), each ^{13}C resonance line would be of the order of 2-3 KH_z in width and each spectrum would contain no discernible spectral definition. To alleviate this problem, high power decoupling has been implemented (approximately 10 times the intensity required to remove the scalar through-bond interactions in solution ^{13}C spectra) to acheive acceptable line narrowing[2].

Additional problems associated with obtaining high resolution ^{13}C spectra in the solid state result from the low sensitivity associated with the dilute ^{13}C nucleus. This is primarily due to the lack of intimacy of the ^{13}C nuclei in the solid, i.e., because of the restrictions imposed in the solid each ^{13}C nucleus cannot interact with one another as frequently as they do in solution and consequently cannot undergo mutual spin flips. The result is intolerably long spin lattice relaxation times, T_1 making data aquisition unfeasable under normal time constraints. To defeat this problem, a technique pioneered by Pines et al.[2], which exploits the large proton population, is used to facilitate relaxation on the rapid proton time scale. In essence this method uses a pulse sequence that induces the protons and carbon nuclei to process at the same frequency in their respective

rotating frames. During this period (milliseconds), which is the cross polarization time, the protons and carbons exchange energy through mutual spin flips and, thus, the rapidly relaxing proton population controls the carbon relaxation process. Typically, spin lattice relaxation times characteristic of cross polarization experiments are of the order of fractions of a second. Additionally the cross polarization process gives rise to ^{13}C signal enhancement due to polarization obtained from the proton population. This results in a fourfold increase in signal-to-noise over the noncross polarization experiment.

Finally, in order to remove a third critical factor responsible for the lack of sufficient resolution in the solid state, i.e., chemical shift anisotropy (CSA). The sample must be spun of frequencies as high as 3-4 KH_z (depending on the magnetic field used) at an angle of 54.7°C. Utilizing this technique with the two mentioned above, ^{13}C cross polarization, magic angle spinning CP-MAS NMR spectroscopy can produce relatively high resolution spectra of crystalline solids and polymers in times comparable to those used to obtain solution spectra[3].

For a complete and detailed account of the CP-MAS process and its application to the study of the structural dynamics of polymers in general the reader is referred to the extensive reviews by Yanoni[4], and Havens and Koenig[5], respectively. More specific information on the applications of ^{13}C CP-MAS NMR to carbohydrates can be found in a recent review by Pfeffer[6].

1.2 Application to Polysaccharides

1.2.1 Studies of cellulose The first CP-MAS ^{13}C studies of cellulose I (native) and mercerized or regenerated cellulose II clearly demonstrated that both crystalline as well as amorphous regions could be defined[7,8]. One of these studies[7] suggested

that the doublet multiplicity of the C-1 and C-4 resonances in cellulose I and II spectra are indicative of unequivalent adjacent anhydroglucose units ascribed to the basic repeat structural unit of anhydrocellobiose. The other report suggested that this splitting was due simply to two magnetically inequivalent environments of glucose monomers[8]. Further refinement of this work by Earl and VanderHart[9] showed that in samples of cellulose I in the form of cotton, ramie, and hydrocellulose there was a clear splitting of the C-1 and C-4 resonances indicative of more than two anhydroglucose residues per unit cell (possibly four) in the crystal structure. In addition, these authors suggest that the broad peaks observed in the spectrum do not originate from paracrystalline regions of the sample, but are attributable to anhydroglucoses on the surface of cellulose elementary fibrils. This conclusion was based on the fact that these broad resonances are missing in spectra of celluloses obtained from Acetobacter xylinium and Valonia ventricosa which have a very high degree of lateral organization and low surface-to-volume ratio.

Maciel et al.[10] clearly addressed the question of order and morphology of cellulose from a single source that was modified mechanically. Their results clearly supported those of Earl and VanderHart[9] that the broad resonances associated with amorphous regions observed in cellulose I samples from biological sources are primarily due to cellulose monomers on the surface. An elegent method for preferentially examining the crystalline or amorphous regions of cellulose has been demonstrated by Horii et al.[12]. This technique exploits the difference in the spin-lattice relaxation times T_1^C in the laboratory frame to suppress the spectrum of one domain while emphasizing the other. In addition, a quantitative assessment of the ratio of amorphous to crystalline cellulose in different cellulosic matrices was demonstrated following a careful evaluation of the critical relaxation process responsible for the spectral responce components.

In a detailed study Dudley et al.[11] examined the ^{13}C CP-MAS spectra of different size cellulose oligomers to determine at what stage the characteristic features of cellulose II appears as the oligomer chain length was increased. From cellotetraose through cellohexaose the authors observed the characteristic C-1 resonance splitting reminiscent of cellulose II, as well as a convergence of the C-1 and C-4 shift with those of cellulose II. Careful integration of the C-1 doublet resonance in the spectrum of cellotetraose demonstrated that these absorptions were clearly in the ratio of 1:1. Since cellotetraose contains only three interglycosidic linkage, alternation of 0.4 dihedral angle pairs should lead to a 2:1 intensity ratio for the C-1 doublet rather than the 1:1 ratio observed. This observation was interpreted as evidence that supports a structure of cellotetraose (and by extension cellulose II) that necessitates two independent chains in the unit cell. A most recent report by Atalla and VanderHart has refined the CP-MAS spectra of native celluloses into two distinct crystalline forms. The authors feel that the spectra of native celluloses have multiplicities that cannot be explained in terms of nonequivalent sites within a unique unit cell[13] as described by Dudley et al.[11]. This work proposed the existence of two different crystalline forms Iα and Iβ which are in different proportions depending on the source of cellulose, e.g., bacterial cellulose such as Acetobacter cellulose containes 60-70% Iα, whereas plant derived cotton contains 60-70% Iβ. These findings have important implications in terms of understanding the mechanism by which plants and microorganisms biosynthetically elaborate cellulose.

Horii et al.[14] has suggested that the chemical shift position of the C-6 resonance in the CP-MAS ^{13}C spectra of carbohydrates is diagnostic for the conformaton of the CH_2OH groups about the exo-cyclic C-C bond. According to X-ray analysis the conformations

about the C-5-C-6 bond is gauche-trans in α-D-glucose and gauche-gauche in α-D-glucose·H_2O, where gauche-trans indicates that the C6-O6 bond is gauche to the C5-O5 bond and trans to the C4-C5 bond. It was therefore assumed for these samples that the low field C-6 resonance at 64.5 ppm for α-D-glucose corresponded to a gauche-trans conformation and the higher field absorption for the C-6 resonance at 61.6 ppm for α-D-glucose·H_2O to a gauche-gauche conformation. A good linear correlation of C-6 shift position with torsion angle χ around the exo-cyclic C-C bonds were obtained with mono and disaccharides. An evaluation of native cellulose in the crystalline state gave good agreement for its assigned trans-gauche conformation. The position of 63.4-63.9 ppm for the C-6 resonance of noncrystalline native cellulose suggest that the CH_2OH group is in the gauche-trans conformation. Regenerated cellulose in the crystalline forms gave a C-6 shift in the low field range consistent with the gauche-trans conformation; however, this interpretation is at odds with previous X-ray studies that say the CH_2OH group exists in both gauche-trans and trans-gauche conformations. The noncrystalline regenerated cellulose has the same shift range and conformation as its crystalline counterpart. Detailed studies[15] of ^{13}C chemical shift assignments for α-D-glucose, α-D-glucose·H_2O, and β-D-glucose, which exploit ^{13}C-^{13}C dipolar interactions in specifically ^{13}C enriched glucose molecules have uncovered differences in the corresponding ring carbon resonance positions for α-D-glucose and α-D-glucose·H_2O that are comparable to the C-6 shift differences mentioned above[14]. Such perturbation that presumably derive from intermolecular interactions could also contribute significantly to the C-6 shift differences for α-D-glucose and α-D-glucose·H_2O attributed to purely conformational preferences. A comparison of the relative positions of the ring carbon resonances in the solid state and those in solution for

the glucose series showed a poor correspondence, again indicating the strong effect of crystal lattice interactions on chemical shift[15]. For a complete review of the ^{13}C CRMAS work being done in the area of cellulose chemistry see the article by Fyfe et al.[16]

1.3 Complex Polysaccharides Matrices

Lodgepole Pine wood was subjected to various mechanical (grinding, ball melting) and chemical processing and the fractions then examined by ^{13}C CP-MAS NMR[17]. Both lignin and carbohydrate fractions gave spectra reflecting their characteristic compositions. The spectra gave indirect evidence for the existence of lignin-carbohydrate complexes since all lignin fractions exhibited carbohydrate signals and vice versa. Spectra of explosion-treated spruce wood suggest that the explosion treatment gives increased crystallinity to the cellulose since their signals are sharper then those obtained from untreated samples[18]. Exploded wood pulp has also been examined by a number of pulse sequences in order to more clearly define the nature of internal lignin-carbohydrate complexes[19]. In addition to quantifying the lignin[20] in this material the criteria of homogeneous proton spin diffusion was used to characterize a single-phase lignin-carbohydrate complex residue derived from cellulase treatment of the pulp[19] In addition doping of the intact wood pulp with paramagnetic Fe^{+3} gave a clear indication that rapid spin diffusion could be efficiently transferred from the Fe^{+3} bound to the carbohydrate to the lignin component[19] ^{13}C CP-MAS has also been effective for monitoring polysaccharide breakdown or disordering in ripening apple cell wall tissue[21]. In this study critical point dried cell walls were examined via various spin lattice relaxation times to estimate changes in polymer mobilities as a function of ripening. Of particular interest were the carbonyl resonances

of the polyuronide which showed 42% drop in proton T_1 over a period of 21 days. These data correlate well with a decrease in fruit tissue firmness over this period of time, indicating that the pectic substances ("glue") within the matrix is breaking down and becoming increasingly disordered[21]. Sodium T_1 values for this tissue show the same trend. Parallel model experiments using cell wall tissue treated with endo-cleaving polygalacturonase enzyme PG II also demonstrated a 57% drop in proton T_1 values for the carbonyl resonance, indicating the effect of polyuronide chain shortening on overall relaxation rates.

^{13}C solid state methodology is becoming useful for determining the primary structure of various polysaccharides and their derivatives[22]. This a particularily important application for highly insoluble derivatives such as xanthan gum and substituted chitosans. Conformational information in (1-3)-β-D-glucans in both noncrystalline and crystalline regions has also been gleaned from its ^{13}C spectra[23]. Following a study of the ^{13}C chemical shifts of various polysaccharides (curdlan, lentinan, and laminaran) in the solid, gel, and solution it was concluded that the high molecular weight glucans adopt mainly a helix form, while the low molecular weight assume a random coil form in addition to the helix in the solid.

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